Genetic Variation through Polymorphism of Blood and Egg White Protein in Three Kinds of Kedu Chickens at Laying Period

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Abstract. Genetic variation of five blood protein loci and three egg white protein loci in three kinds of Kedu chicken, namely the black skin and black feather (BB), black skin and white feather (BW) and white skin and white feather (WW) were investigated using polyacrilamide gel electrophoresis. The result showed that five blood loci (pre-albumin, albumin, post-transferin, transferin and hemoglobin) and three egg white loci (lysozim, ovalbumin and conalbumin) were found to be polymorphic in three kinds of Kedu chicken. BW has average higher heterozygosity than BB and WW. The result was shown in blood and egg white protein polymorphism. There was no difference on blood and egg white protein in the genetic variation. The average of heterozygosity of Kedu chicken of high production and low production was not different. Genetic distance among three populations of Kedu chicken showed that Kedu chicken BW was closer to WW than to BB.

Key Words: Kedu chicken, protein polymorphism, blood, egg white, genetic variation

Introduction

Kedu chicken is one of famous local chickens in Indonesia and has high potential and economic value to produce egg and meat. Kedu chicken, especially those with black skin and black feather were used by people in some ceremonial activities. Farmers still use traditional method in rearing Kedu chicken. In fact, there are many uncontrolled crossbreeding of Kedu chicken causing high genotype variation that leads to different productivity (Johari et al., 2008). Hence, there is attention to increase the genetic quality to keep preservation (Mahfudz et al., 2005 and Johari et al., 2008). One way to increase genetic quality is continuing selection of Kedu chicken by choosing the high potential to improve (Mahfudz et al., 2005 and Johari et al., 2008).

One of the economic potential of Kedu Chicken is egg production (Mahfudz et al., 2005). High or low egg production depends on genetic variation, because productivity of animals depends on environment and genetic factor (Johari et al., 2008). This is very important to know, because it can help to select the highly productive animal and low productivity of chicken (Mahfudz et al., 2005; Ismoyowati, 2008 and Johari et al., 2008).

The genetic variation is one of the bases to know the change of selection value in the population (Li Chan and Kim, 2008; Miguel et al., 2005 and Setioko et al., 2005; Zang et al, 2002[ab]). The genetic variation can show with allele characters from the specific locus of liquid or body tissue like blood, egg white and yolk (Muzani et al., 2005; Praseto and Ketaren, 2005).

Blood and egg white have different locus of protein (Mahfudz et al., 2005; Ismoyowati, 2008 and Johari et al., 2008). The protein loci in blood are pre-albumin, albumin, pre-tranferin, transferin and haemoglobin (Warwick et al., 1990; Inafuku et al., 1998 and Johari et al., 2008) and loci of protein from egg white are ovalbumin, conalbumin and lysozim (Johari et al., 2008) and there are still many others. That statement can be explained that blood and egg white have different potential of protein content, but both of them can be used to study the genetic variation (Mahfudz et al., 2005; Johari et al., 2008). Consequently, it is very

important to know the genetic variation of blood and egg white protein and genetic variation between high and low egg production of three kinds of Kedu chickens (Mahfudz et al., 2005 and Johari et al., 2008).

This study was conducted to clarify the gene constitutions in three kinds of Kedu chickens, to know the differences of blood and egg white protein analysis as shown from genetic variation in three kinds of Kedu chickens, and to know the difference between Kedu chickens that have high and low egg production.

Materials and Methods

Blood and egg white were collected from 45 Kedu chickens of black skin and black feather (BB=15 birds), the black skin and white feather (BW=15 birds), and the white skin and white feather (WW=15 birds). Blood and egg white from each kind of Kedu chickens were divided into 2, namely 1) high egg production and 2) low egg production. The blood protein polymorphisms at 5 loci obtained from the blood samples and egg white samples were used to analyze the egg white protein polymorphism at 3 loci obtained from the egg white samples. Egg production was quantified by hen day production, using the following formula:

$$HDP = \frac{\sum egg\ \ production}{rearing\ \ of\ \ chicken}\ x\ 100$$

Genetic variability within the population was quantified by measuring the average heterozygosity, *H*. The average heterozygosity was estimated from the expected proportion of heterozygosity per locus, using the following formula (Nei, 1978 and Johari et al., 2008) :

$$H = \frac{1 - \sum_{i=1}^{m} x_1^2}{r}$$

Where x_1 is the frequency of the allele at a locus, m is the number of allele, and r is the number of locus. The genetic distance between population and average heterozygosity were calculated using the DISPAN computer (Johari et al., 2008).

Results and Discussion

Blood groups and blood protein polymorphism

Table 1 shows the distribution of phenotypes and gene frequencies of blood protein of three kinds of Kedu chickens (5 loci). Out of 5 loci, P-Alb, Alb, Tf, P-Tf, Hb were polymorphic. BB and WW Kedu chickens have the same frequency of P-Alb^B (0.75), higher than BW (0.71). This species was detected to have the highest difference of allele frequency in the P-Alb, Alb, Tf, P-Tf, and Hb. For example in the albumin locus, in BB allele Alb^A frequency was 0.25, Al^B 0.75. These alleles were found in BW and WW with frequency of 0.29 and 0.71, and 0.25 and 0.75.

In BB, the Tf ^{C} locus was not detected due to the smaller number of population (10) than BW and WW (12). The rare variant of allele Hb^A was found only in BB population with frequency of 0.05. In BW and WW, this allele was not detected; even the number of chicken was higher than BB. In general, the allele frequency of BB is different from that in WW and BW when more loci were examined. The similar allele frequency in BW and WW populations was Hb locus.

The genetic variability of three populations of Kedu chicken is shown in Table 2. The estimated *H* values were from 0.2844 to 0.3934. The mean heterozygozity in BB was lower (0.2844) than those in BW (0.3934) and WW (0.3348). This result suggests that BW was crossbred between BB and WW.

Genetic distance from three kinds of Kedu chickens was shown in Figure 1. The longest genetic distance was observed between BW and WW with BB. Cluster analysis revealed BW and WW population from one cluster, while BB population from other cluster. This statement

was supported that BB has much more homozygote allele than BW and WW.

Egg white groups

The gene frequency at the egg white group lisozim in three kinds of Kedu chicken is shown in Table 3. The frequency of genes of A allele was considerably higher than B allele. In the BW, the frequency of A allele (0.58) was lower than those of BW (0.90) and WW (0.92). The gene frequency in the ovalbumin and conalbumin of A allele was considerable higher than B allele.

The genetic difference in three kinds of Kedu chickens evaluated through the coefficient of average heterozygosity per individual could be seen in the Table 3. The *H* values were estimated from 0.2518 to 0.4742. The average heterozygozity of BB was lower (0.2518) than those in BW (0.4702) but higher than those of WW (0.3804). This result was similar to heterozigosity of Kedu chicken electrophoresis egg white protein (Table 1). This was due to several egg whites and blood protein locus showing that BB and WW had allele phenotypic characteristic to be more homozygous than those of BW. According to Sopiyana et al. (2006) and Johari et al. (2008), inbreeding could increase the proportion of gene pairs that are homologous and decrease the proportion. Metric of the genetic distance from calculation of gene frequency in egg white protein is presented in Table 4. The close genetic distance from three kinds of Kedu chicken is BW and WW 0.0074. BW and WW have genetic distance of 0.0081. This statement was supported the fact that BB has much more homozygote allele than BW and WW.

Those cluster of data analysis followed DISPAN program. The dendogram (Figure 2) showed that BW and WW formed the same cluster. It seems that BB was from another cluster.

Heterozigosity

Heterozigosity from three kinds of Kedu chicken was shown by blood and egg white protein that can be seen in Table 5. Heterozigosity from three kinds of Kedu chickens contained in blood and egg white protein was different but the high value was same. According to Warwick et al. (1990) and Johari et al. (2008), blood locus were transfertin, albumin, hemoglobin and enzymes; while egg white protein locus were ovalbumin, conalbumin, ovomukoid, and lisozym. Following the statistical analysis, the heterozigosity between blood and egg white protein was not significant (P>0.05). This means that blood and egg white protein can be used to show genetic variation by electrophoresis. This result is similar to the report of Mahfudz et al. (2005)

Table 1. Gene frequencies of the blood group and blood protein loci of three kinds of Kedu chickens and average heterozygosity

Locus	Allele	Population		
		BB (N=10)	BW (N=12)	WW (N=12)
P-Albumin	A	0.25	0.29	0.25
	B	0.75	0.71	0.75
Albumin	A	0.75	0.54	0.71
	B	0.25	0.46	0.29
Transferin	A	0.15	0.17	0.04
	B	0.85	0.50	0.29
	C		0.33	0.67
P-Transferin	F	0.90	0.83	0.83
	S	0.10	0.17	0.17
Hemoglobin	A	0.05	0.00	0.00
	B	0.95	1.00	1.00
Average heterozygosity		0.2844	0.3934	0.3348

Table 2. Genetic distance between every pair of the Kedu chicken population, distance was calculated from gene frequencies at 3 blood group and 6 blood protein loci

Population	1	2	3
BB	0.0000		
BW	00.0014	0.0000	
WW	0.0762	0.0114	0.0000

Table 3. Gene frequencies of the egg white group and egg white protein loci of three kinds of Kedu chicken and the average heterozygosity

Locus	Allele	Population		
		BB (N=10)	BW (N=12)	WW (N=12)
Lysozim	A	0.90	0.58	0.92
	B	0.10	0.42	0.08
Ovalubumin	A	0.80	0.75	0.71
	B	0.20	0.25	0.29
Canalbumin	A	0.90	0.67	0.79
	B	0.10	0.33	0.21
Average heterozygosity		0.2518	0.4742	0.3804

Table 4. Genetic distance between every pair of Kedu chicken population calculated from gene frequencies at 3 blood group and 6 blood protein loci

Population	1	2	3
BB	0.0000		
BW	0.0168	0.0000	
WW	0.0081	0.0074	0.0000

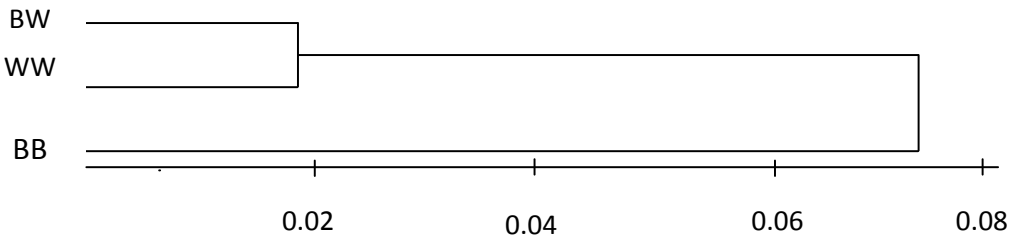

Figure 1. Dendogram of Kedu chicken

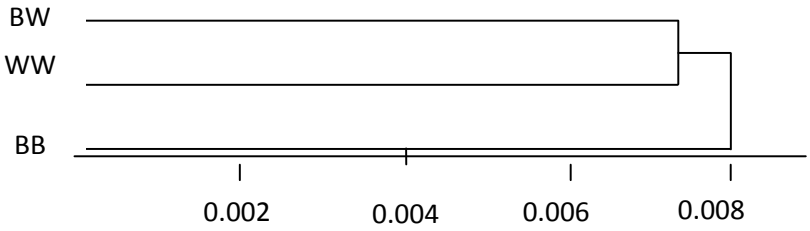

Figure 2. Dendogram of Kedu chicken by egg white protein

Table 5. Heterozigosity between blood and egg white protein

Population	Treatment		Average	Significance
	Blood	Egg White		
BB	0.2844	0.2518	0.2681	ns
BW	0.3934	0.4743	0.4338	ns
WW	0.3348	0.3804	0.3576	ns

ns = non significant (P>0.05)

Table 6. Heterozigosity of Kedu chicken between high production (HP) and low production (LP)

Population	HP		LP	
	∑Animal	$\overline{H}$	∑Animal	$\overline{H}$
BB	6	0.1903	4	0.3936
BW	7	0.3916	5	0.2150
WW	6	0.3318	6	0.3699

Table 7. Heterozigosity between high production (HP) and low production (LP)

Population	Treatment		Average	Significance
	Blood	Egg White		
BB	0.1903	0.3936	0.2919	ns
BW	0.3916	0.2150	0.3033	ns
WW	0.3318	0.3699	0.3508	ns

ns = non significant (P>0.05)

Table 8. Heterozigosity of Kedu chicken between high production (HP) and low production (LP)

Population	HP		LP	
	∑Animal	$\overline{H}$	∑Animal	$\overline{H}$
BB	6	0.3217	4	0.1877
BW	7	0.5299	5	0.4417
WW	6	0.3949	6	0.3666

Table 9. Heterozigosity between High Production (HP) and Low Production (LP)

Population	Treatment		Average	Significance
	Blood	Egg White		
BB	0.3217	0.1877	0.3003	ns
BW	0.5299	0.4417	0.4858	ns
WW	0.3949	0.3666	0.3807	ns

ns = non significant (P>0.05)

and Johari et al. (2008) that blood and white protein can be used as genetic variation by electrophoresis.

Heterozigosity of high egg production

The average of heterozigosity on protein locus was shown in blood and egg white protein. The high and low egg production are shown in Table 6 and 7.

The heterozigosity of blood and egg white protein of high or low production did not show significant (P>0.05) difference (Table 8 and 9). However, it might not reflect the true situation because the number of population used in this experiment was insufficient.

According to Mahfudz et al., (2005) and Li Chan and Kim (2008) factors influencing the egg

production were age, light and single gen. Gene which influences egg production was dwarfism gene (Miguel et al., 2005 and Johari et al., 2008).

Conclusions

Average heterozigosity of three kinds of BW Kedu chickens was the highest. followed by WW and BB. Kedu chicken with low and high productivity was not significantly different on genetic variance. Blood and egg polymorphism has potential to determine the genetic variation of chickens.

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